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Plasma Glucose, Protein and Cholesterol Levels of Chicks or Birds Maintained on Pawpaw (*Carica papaya*) Seed Containing Diet

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Abstract: Proximate analysis of dehusked *Carica papaya* seeds indicated the presence of the following constituents: Ash, 6.0%; fat, 14%; fiber, 18%; protein 23.1% and carbohydrates, 28.6%. Plasma assays of glucose, protein and cholesterol in the blood of birds maintained on the diet were determined and compared with the control. The birds were grouped into A (control), B (Intermediate group) and group C (test group). The parameters assayed exhibited the following results: Group A (190.05±1.61) range 42.70 mg/dl; 6.39±0.15) range 0.80 g/dl and (107.68±12.50), range 42.70 mg/dl; for glucose, protein and cholesterol respectively. For the intermediate group (group B), the following were derived for the parameters: (194.90±1.60) range 3.20 mg/dl; 6.41±0.05 (range 0.4 mg/dl) and 114.13±18.30 (range 25.0 mg/dl). For group C (test group), the following values were derived: 201.75±5.50 (range 19.50 mg/dl) 6.40±0.20 (range 0.80 mg/dl) and 104.98±3.30 (range 14.10 mg/dl) respectively. The average weight gain for the respective groups were: 268±0.18 (range 0.23 kg) for group A; 278±0.04 (range 0.13 kg) for group B and 2.96±0.08 (range 0.34 kg) for group C. The amount of food consumed were as follows: 5.13±1.40 (range 4.5 kg), 6.17±2.60 (range 6.4 kg) and 7.70±3.8 (range 8.8 kg) respectively; while the feed efficiency values were as follows: 0.37±0.03 (range 0.22), 0.33±0.01 (range 0.31) and 0.31±0.06 (range 0.49 respectively. Statistical analysis at $p \geq 0.5$ indicated no significant difference in the means of the parameters investigated. Dehusked seeds of *Carica papaya* could be nutritionally, economically and therapeutically beneficial in poultry farming and management.

Key words: *Carica papaya*, plasma glucose, protein, cholesterol

INTRODUCTION

The quest for alternative sources of energy has been the paramount occupation of scientists all over the universe to sustain biological life in this global architecture. The source of energy to animal life has facilitated researches in different fields of knowledge as a bid to reducing cost and achieving longer life span. It is an obvious fact that man depends on plants for food and medicinal uses. *Carica papaya*, otherwise known as pawpaw is a tropical American plant (Purseglove, 1974). It has spread to all tropical and sub-tropical countries (Samson, 1980). Glucose and other monosaccharides are primary sources of energy to animal cells. The nerves, the lung tissues and the brain use glucose as their source of fuel. The level of blood glucose depends on the balance between the intake of carbohydrates and the endogenous glucose synthesis and release by the liver on one hand and storage, utilization and excretion on the other (Baron *et al*., 1993). The blood of chicken contains about 200-250 mg of glucose per 100 ml of blood, which originates from digested carbohydrates. Protein makes up about three to a quarter of the body solids which include the structural proteins, enzymes, nucleoproteins, protein in the form of hemoglobin, proteins of muscles and other forms that perform specific functions. Plasma contain over one hundred types of proteins with great diversity of physiochemical characteristics and physiological roles (Baron *et al*., 1993). Most of the plasma proteins consists of albumin, globulin and fibrinogen. At physiological pH of 7.4; plasma proteins maintain the water balance of blood, transportation and storage of a large variety of ligands such as fatty acids, calcium, bilirubin and hormones such as thyroxine.

Plasma cholesterol is normally assayed because of the strong correlation between its high levels in the blood and the incidence of cardiovascular diseases in humans (Nelson and Cox, 2005). It is the precursor of bile acids and steroid hormones in the body such as the corticosteroids, sex hormones and vitamin D. It is typically a product of animal metabolism (Ganong, 1991; Baron *et al*., 1993). Cholesterol can be obtained from the diet or can be synthesized *de novo*. Approximately, about 1 g/day of cholesterol of the body arises by synthesis where as 0.3 g/day is provided by diet. Atherosclerosis is characterized by the deposition of cholesterol esters and other lipids in the connective tissues of the arterial walls. The rabbit, pig and cat are resistant. Cholesterol is an amphipathic lipid and as such, an essential structural component of membranes. *Carica papaya* is one of the genera and is cultivated throughout the tropics. The pawpaw fruit is a large fleshy, hollow berry, weighing 0.5-2.0 kg per fruit. Based on the chemical analysis, the fruit contains 88% water, 10% sugar

(carbohydrate), 0.5% proteins, 0.1% fat, 0.7% fiber, 0.6% oil and 0.1% acids (Purseglove, 1974). It is rich in vitamins A and C (Ekeke *et al*., 2001). *Carica papaya* contains many biologically active compounds which include Chymopapain and papain, which aid in digestion and milk clotting (Foyet, 1972). It has been used industrially in brewing and wine making. *Carica papaya* has been estimated to contain the following compounds: alkaloids, butanoic acid, methylbutanoate, carpaine, flavonoids, pseudo carpaine, papain, chymopapain-a and -b, tannins, α-linolenic acid, nicotine, benzylisothiocyanate, etc. Papain latex is probably of more interest to livestock producers as it is applied as anti-helminthic (Suhalla *et al*., 1994). Water extracts of papaya seeds decreased *Ascardia galli* infections in chicks by 41.7% (Kumar *et al*., 1991). *Carica papaya* extracts have been used to cure several forms of ailments in several parts of the globe, depending on the part of the tree, sex, age, extraction method and chiefly, the type of ailment.

The research work aims at providing alternative source of nutrient energy for poultry animals and equally, the reduction in the cost effectiveness of animal nutrition and husbandry.

MATERIALS AND METHODS

Materials used in this work included an array of laboratory reagents and equipment for analysis, $CuSO_4$, H_2SO_4, 99% Ethanol, Potassium sodium tartarate, Dihydroxybutanedioate, water, Glucose oxidase, glucometer, Peroxidase, Mutarotase, 4-Aminoantipyrine, p-Hydroxybenzene sulphonate, Cholesterol oxidase, all of analytical grade, purchased from Sigma Biochemicals, London.

Dietary materials: Powdered maize, powdered cake, fish meal, Palm Kernel Cake (PKC), bone meal, wheat offal, Premix, Methionine, Lysine, Pawpaw seeds (dehusked) and salt.

Drugs: Intraoccular, Gamboro, Lasota, Multivitallite, etc.

Proximate analysis: The recommended methods of the Association of Official Analytical Chemists (AOAC, 1990) were used for the determination of moisture, ash, crude lipid, crude fibre and nitrogen content.

Determination of crude lipid: Two grams (2 g) in triplicate of dried sample were weighed into the porous timble and its mouth plugged with cotton wool. The timble placed in the extraction chamber which was suspended above the weighed receiving flask containing petroleum ether (bp 40-60°C) and below a condenser. The flask was heated for eight hours to extract the crude lipid. The flask containing the crude was disconnected from the Soxhlet, the oven dried at 100°C for 30 min, cooled in a dessicator and weighed. The difference in weight is expressed as percentage of crude lipid content.

Determination of crude fiber: The crude fiber was determined as the organic residue left after treating the sample under standard conditions with petroleum ether and then boiled in 1.25% H_2SO_4 (w/v) and 1.25% NaOH (w/v) solutions. The residue after crude lipid extraction was used for this assay. Crude fiber content was expressed as percentage loss in weight on ignition.

Determination of nitrogen content of Pawpaw: Micro-Kjeldahl method was used to determine the nitrogen content of the sample. Two grams of dried powdered sample was placed in a 100 cm^3 Kjeldahl digestion flask. A kjeldahl digestion tablet and 10 ml of concentrated tetraoxosulphate (vi) acid were added and the sample digested gently until frotting stopped. The mixture was boiled until the digest become clear. The content was filtered into a 100 ml volumetric flask and made up to 100 ml with distilled water. 10 ml of the aliquot solution and 20 ml of 45% NaOH solution were put into a distillation flask and steam distilled. The ammonia liberated was collected over 50 ml, 20% boric acid mixed indicator solution, cooled and titrated with standard 0.01 M HCl solution. Blank determination was carried out in similar manner.

Determination of crude protein and available carbohydrate: Crude protein was estimated by multiplying the sample percentage nitrogen content by a factor of 6.25. Available carbohydrate was calculated by the difference method by subtracting the total or sum of crude protein, crude lipid, crude fiber and ash from the 100% DW sample.

Design of experiment: A total of twenty birds of age two months (broilers) were used. They were divided into five in each group, kept in a room of dimension 1.5 m^2. The birds were assumed to have been on commercial diet (Starter mash) for few weeks and the formulated diet for few more weeks prior to purchase. After purchase, the birds were maintained on a commercial feed (Broiler finisher) for one week. Then, the birds were fed as follows: Group A (control) were fed diet containing 64.33 g of maize. Group B (Intermediate group) were fed diet containing pawpaw seeds, substituting ¼ of the maize component of the control diet. Group C were fed diet containing pawpaw seeds substituting ½ of the maize in the control. Water was given to the birds *ad libidum*. The individual weights of the birds were taken prior to the commencement of the dietary substitution of maize with the dehusked pawpaw seeds.

Ration formulation: This was carried out to determine the nutrient requirement of the stock whose ration is to be formulated, to access the nutrient content and availability of foodstuff to be used. Ration formulation was carried out by the Pearson Square Method (Obioha, 1992).

Metabolizable energy of pawpaw: The energy value of pawpaw seed is calculated by the Atwater factors of 4, 9 and 4 as reported by Onyeike and Osuji (2003). The value of protein content is multiplied by 4; that of lipid by 9 and that of total carbohydrate by 4. The sum of these values is expressed in Kcal/100 g sample. The metabolizable energy of pawpaw seed is 332.8 Kcal/g. The wet weight of one dehusked pawpaw seed is 0.0075 g and a husked seed weighed 0.01 g.

Metabolizable energy of maize: The metabolizable energy of maize was calculated as shown below. According to Obioha (1992) maize has 10% protein, 4.6% fat and 83% carbohydrate. Hence, carbohydrate = 83 x 4 = 332; fat = 4.6 x 9 = 41.4; protein = 10 x 4 = 40; Total = 413.4 Kcal/g.

Composition of diet fed on chicks or birds

Materials	Control (A) %	Group (B) %	Group (C) %
Groundnut cake	20.63	20.63	20.63
Palm Kernel Cake (PKC)	4.42	4.42	4.42
Fish meal	4.40	4.42	4.42
Maize	64.33	48.25	32.17
Pawpaw	-	16.08	32.17
Bone meal	2.00	2.00	2.00
Wheat offal	2.00	2.00	2.00
Premix	0.50	0.50	0.50
Methionine	0.50	0.50	0.50

Blood sample collection: Blood samples were collected from each of the birds of the three respective groups. This was done by using a syringe to draw 1.0 ml of blood from the veins of the wing side (right wing).
The blood samples were collected using anticoagulant bottle or container. The samples were later centrifuged to obtain the plasma used in the test.

Quantitative determination of total plasma glucose: This was determined using glucose oxidase test method.

Quantitative determination of total plasma protein: This was done by the Biuret method of Lowry *et al.* (1951).

Quantitative determination of total plasma cholesterol: This was carried out by the methods of Holvey (1972).

RESULTS

The results of the various assays are shown in Table 1-6.

Table 1: Proximate analysis of pawpaw seeds

Parameter	Percent (%)	Parameter	Percent (%)
Moisture	10.5	Ash	6.0
Fat	14.0	Protein	23.1
Fiber	18.0	Carbohydrates	28.6

Table 2: Plasma glucose, protein and cholesterol concentrations of group A (Control)

Group	Glucose (mg/dl)	Protein (mg/dl)	Cholesterol (mg/dl)
A_1	198.10	6.35	119.00
A_2	165.00	6.40	105.80
A_3	189.40	6.20	93.30
A_4	207.70	6.60	121.60
Average	190.05±24.40	6.39±0.15	10768±12.50
Range	42.7	0.80	28.30

Table 3: Plasma glucose, protein and cholesterol concentrations of the intermediate group (B)

Group	Glucose (mg/dl)	Protein (mg/dl)	Cholesterol (mg/dl)
B_1	194.90	6.20	98.30
B_2	196.50	6.60	116.60
B_3	193.30	6.25	123.30
B_4	194.90	6.60	118.30
Average	194.90±1.60	6.41±0.05	114.13±18.30
Range	3.30	0.40	25.00

Table 4: Plasma glucose, protein and cholesterol concentrations of group (C), the test group

Group	Glucose (mg/dl)	Protein (mg/dl)	Cholesterol (mg/dl)
C_1	199.00	6.60	111.60
C_2	213.00	6.00	97.50
C_3	201.50	6.25	100.80
C_4	193.50	6.60	110.30
Average	201.75±5.50	6.40±0.20	104.98±3.30
Range	19.50	0.80	14.10

DISCUSSION

From the statistical analyses of all the results obtained for plasma glucose, protein and cholesterol levels in the control group, intermediate group and the test group fed pawpaw seed containing diet; there is virtually no significant difference in the mean (X) of the parameters investigated. The relative constant plasma protein levels of the chicks or birds in the control groups, intermediate and test groups could be as a result of adequate utilization of the dietary materials in the groups. According to Anosike (1994), the requirements for dietary protein arises from the requirements for certain amino acids which are considered essential for the body. This requirement was met by the constant level of proteins in the diet fed to the animals in the three groups. The Metabolizable Energy (ME) of the diet fed the experimental animals indicated a value of 580.7 kcal/g.

Table 5: Summary of parameters and values

Group	Glucose (mg/dl)	Protein (mg/dl)	Cholesterol (mg/dl)
A	190.05±24.40; Range = 42.70	6.39±0.15; Range = 0.80	107.68±12.50; Range = 28.30
B	194.90±1.60; Range = 3.30	6.41±0.05; Range = 0.40	114.13±18.30; Range = 25.00
C	201.75±5.50; Range = 19.50	6.40±0.20; Range = 0.80	104.98±3.30; Range = 14.10

Table 6: Weight gain per bird per group

Group	Weight (kg)	Group	Weight (kg)	Group	Weight (kg)
A_1	2.73	B_1	2.75	C_1	2.85
A_2	2.72	B_2	2.84	C_2	3.11
A_3	2.54	B_3	2.71	C_3	3.10
A_4	2.77	B_4	2.77	C_4	2.77
Average	2.69±0.18	2.78±0.04		2.96±0.08	
Range	0.28 kg	0.13 kg		0.34 kg	

This calculation was eventuated by the observation of Neishein *et al.* (1972) that the efficiency of feed utilization increases as the energy of the diet increases. Though maize has 83% carbohydrate, while pawpaw has 39.20%, the experimental animals have half the carbohydrate content of maize, that is 41.15 g in addition to that of pawpaw. It has been observed that feed efficiency does not depend on carbohydrate content of the feed alone (Obioha, 1992). The relative constant level of plasma cholesterol as seen in the chicks studied in both the control and experimental groups could be attributed to both hereditary and dietary factors, since the chicks came from the same stock, probably; there are not much variations in their endogenous biosynthesis of cholesterol. This is in accordance with the observation of Mayes (1993), that hereditary factors play the greatest role in determining an individual blood cholesterol concentration. The level of blood or plasma cholesterol depends on a gamut of factors especially the dietary intake of saturated fatty acids with polyunsaturated and monounsaturated fatty acids, determines individual's blood cholesterol. Pawpaw seeds as obtained, are also rich in lipids. These exogenous sources of cholesterol. Inhibit exogenous biosynthesis (Guyton, 1991). Considering the weight gain in the groups, it seems that the efficiency in feed utilization of the birds is accountable for this. Naturally, animals tend to loose weight when inadequate source of metabolizable energy is efficiently supplied. However, the general performance of the animals (birds) in the test group is a clear indication of non-toxicity of the pawpaw seeds. Specifically, toxic substances tend to inhibit protein synthesis We recommend that *Carica papaya* seeds can comfortably and confidently be incorporated into poultry feeds. From the results obtained, the birds that were on pawpaw seed-containing diets were vibrant and healthy, displaying voracious appetite for the diet. One of the benefits of this study is that pawpaw seeds that were neglected or otherwise regarded as waste, may prove useful in animal sciences and husbandry for human nutrition and medication. Only 50% substitution for maize, produced this excellent finding. The reduction in cost effectiveness of feeds and the improvement on the health of the birds is an excellent breakthrough for poultry farmers if the findings are successfully applied.

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